

# Methods and Approaches for Assessing Immunotoxicity: An Overview

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The goals of the National Toxicology Program as they relate to immunological evaluation in toxicity assessment are discussed. The advantages of immune function assays for defining cellular injury as subtoxic levels following exposure to general or immunocyte specific chemical toxicants are proposed. A comprehensive screening panel of immune function and host resistance assays is presented in the context of an NIEHS approach for immunotoxicity assessment and methods selection. A second panel for defining the mechanism underlying immunological injury was also described. Studies utilizing these methods and approaches are described in companion papers by our group.

## Introduction

A major objective of the National Toxicology Program (NTP) and a major effort at NIEHS is to examine a variety of *in vivo* and *in vitro* approaches to assess toxicity as well as newer research disciplines for evaluating chemicals of environmental concern. Immunology represents one of the disciplines that the NTP is employing to examine possible target organ toxicity as well as generalized toxicity at the cellular and molecular level. Furthermore, the effects of chemicals on host defense to bacterial, viral, fungal and parasitic diseases as well as the surveillance and destruction of neoplastically transformed cells are simultaneously being evaluated. It is our goal at NIEHS to apply systematically the disciplines of immunology and microbiology in a comprehensive manner to study immunotoxicity and altered host resis-

tance and to define a panel of sensitive assays to screen for chemical-induced changes in immunological and host resistance parameters. Extensive studies conducted at NIEHS have suggested that the route and time of exposure relative to the maturational development of the immune system should be of paramount consideration in the design of an evaluation protocol. Several studies have demonstrated that pre- and postnatal exposure to certain chemicals (e.g., TCDD or DES) during the maturation of the thymus-dependent elements of the immune system often produces more profound immunological effects than adult exposure (1) or produce long-lasting effects throughout adult life (2). Thus, the focus of the discipline of immunology on the evaluation of chemical toxicities may provide beneficial information to facilitate better human risk assessment.

Preliminary data indicate that examination of immune function will add an extra level of sensitivity and safety for defining toxicity at the cellular level. The increased sensitivity of the immune system for detecting cellular toxicity may result from its rapid proliferation or the dynamic and

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highly regulated nature of its cellular components. This concept is supported by human clinical chemotherapy studies in which cells of the reticuloendothelial system (RES) are among the most frequent target cells for chemical injury. The functional integrity of cell-mediated immunity (CMI) or the quantitation of bone marrow progenitor elements following chemotherapy is a popular means of regulating drug dosage.

## Comprehensive Panel for Evaluating the Immunobiologic Effects

A test for evaluating immunologic effects should meet several criteria if it is to be considered useful and definitive. The test should result in data which can be extrapolated to the human experience and adaptable to practical considerations such as expense, simplicity, time required for completion, reproducibility, uniformity and application to routine subchronic or chronic toxicology studies. Certain compromises are associated with the development of screening techniques. Ideally, immunologic assays that will identify and assess the risk potential of a chemical or drug are desirable; however, in general, no single assay can accomplish this task, and as such, a panel of selected assays that have been

validated in experimental rodent models and human clinical studies is recommended.

Currently, a wide variety of assays is being utilized in the Immunology Program at the National Institute of Environmental Health Sciences to assess immunologic and host resistance alterations following chemical exposure (Table 1). This is not to suggest that it is necessary or even desirable for evaluation laboratories to employ such a comprehensive assay panel. Rather, at our present stage of knowledge, it is reasonable that a few laboratories perform a comprehensive panel of assays concurrently to determine the relative simplicity, reproducibility and predictability of these methods for detecting altered immunological and host resistance functions. Some of these methods will undoubtedly be selected to screen new chemicals and drugs for immunotoxicological potential. The detailed methodologies of these assays as performed at NIEHS have been described elsewhere in detail (3). These procedures were selected because they are reproducible and easily standardized. During the method selection phase of our research, major emphasis has been placed on assays that could be automated, routinized or require only microquantitates of cells or body fluids. These procedures involve the evaluation of pathotoxicology indicators, host resistance models, delayed-type hypersensitivity, cell-mediated immu-

Table 1. Comprehensive screening panel for defining immune alterations currently being evaluated at NIEHS.

Parameter	Procedure performed
Pathotoxicology	Hematology profile (hemoglobin, red blood cell count, white blood cell count, differential) Liver chemistries (SGPT, triglycerides, cholesterol) Serum proteins (albumin, globulin, A/G, total proteins) Weights (body, spleen, thymus, liver, kidney, heart, lung, brain) Histology (liver, thymus, lung, kidney, spleen)
Host resistance	Tumor assays (tumor cell challenge TD <sub>10-20</sub> and radiometric tumor mass) <i>Listeria monocytogenes</i> LD <sub>10-20</sub> challenge Endotoxin hypersensitivity-LD <sub>10-20</sub> Expulsion of <i>Trichinella spiralis</i>
Delayed hypersensitivity	Radiometric assay with T-cell-dependent antigen
Lymphocyte proliferation	One-way mixed leukocyte culture Mitogens (PHA, Con A, LPS)
Humoral immunity	Immunoglobulin levels (IgG, IgM, IgA) Antibody response to T-dependent and T-independent antigens
Macrophage function	Resident peritoneal cell numbers and nonspecific esterase staining Phagocytosis of <sup>51</sup> Cr-SRBC Lysosomal enzymes (5'-nucleotidase, acid phosphatase, leucine amino peptidase) Cytostasis of tumor target cells Cytolysis of tumor target cells RES clearance using <sup>125</sup> I-trioleion
Bone marrow colony forming units	CFU-S (multipotent, hematopoietic stem cells) CFU-GM (granulocyte/macrophage progenitor) CFU-E (erythrocytes progenitor) Cellularity <sup>59</sup> Iron incorporation in bone marrow

**Table 2. Procedures to define the mechanism of chemically induced immune alterations.**

Altered parameter	Further procedures to perform
Pathotoxicology	Bioaccumulation study Hormone levels Pair-feeding
Host resistance	Virus challenge Staphylococcus or Streptococcus challenge (B-cell-dependent)
Cell-mediated immunity	DHR using T-cell independent antigen Suppressor cell studies with mitogens and cocultures Helper cell studies Spontaneous cytotoxicity Lymphokine production
Antibody-mediated immunity	Mishell-Dutton assay and cocultures Local production of antibody Titer of serum antibody-T-cell-independent antigen
Bone marrow toxicity	Serum levels of colony stimulating factor (CSF) CSF and prostaglandin synthesis by macrophages and bone marrow stromal cells

nity, humoral-mediated immunity, macrophage function and bone marrow progenitor cells. Attempts are being made to correlate changes in immune function with altered host resistance.

If the *in vivo* and *in vitro* data obtained from such carefully planned studies using these screening procedures are negative, there can be reasonable confidence in the safety of the drug or chemical under the conditions and dosages defined.

A major limitation in risk assessment has involved extrapolation of dose response curves from effect to no-effect levels or from rodent model systems to

humans. However, if conservative extrapolations are made by using data from appropriate assays, the most relevant and accurate estimate possible will be obtained. At present the methods listed are in the validation and selection stage for later application of some of these to routine toxicity assessment when immunology studies are indicated.

If warranted by preliminary data, additional tests can be used to examine the mechanisms by which a particular chemical or drug alters immune function (Table 2). These assays can provide additional information regarding mechanisms of immunotoxicity and may provide a means by which to circumvent or abolish the undesired effects of the agent. If the pathophysiologic mechanism responsible for the effect or the target cell is defined, possibly new analogs of the chemical or drug can be synthesized which produce the desirable effects but not the undesirable ones (e.g., synthetic penicillin).

The following papers in this session describe, in detail, methods and approaches utilized by leading laboratories in this rapidly developing field.

## REFERENCES

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